



UNIVERSITI PUTRA MALAYSIA

**ISOLATION AND CHARACTERIZATION OF MICROSATELLITE LOCI
FROM THE GIANT FRESHWATER PRAWN (*MACROBRACHIUM
ROSENBERGII*)**

SEE LENG MIN

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ROSENBERGII*)**

By

SEE LENG MIN

**Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia,
in Fulfilment of the Requirement for the Degree of Master of Science**

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Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Master of Science

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September 2008

Chairman: Professor Tan Soon Guan, PhD

Faculty: Science

The giant freshwater prawn, *Macrobrachium rosenbergii*, or locally known as udang galah has become the most popular freshwater prawn for commercial culture and a significant cash crop for many poor farmers throughout Asia and the Pacific region. Ninety microsatellite repeat sequences were successfully isolated from *M. rosenbergii* using the 5' anchored-PCR technique. BLAST analysis of the microsatellite marker flanking regions showed similarities towards expressed sequence tags (ESTs) in aquatic species. Sixty-two microsatellite primer pairs were designed with 29 perfect microsatellites, four were imperfect or interrupted microsatellites and the rest were compound microsatellites. Of these 62 single locus DNA microsatellite markers, 24 showed polymorphisms in the giant freshwater prawns of which four loci had dinucleotide, 16 trinucleotide, three tetranucleotide and one pentanucleotide core repeat units. Nine microsatellite primer pairs from the

green-lipped mussel (*Perna viridis*) were successful in cross-amplifying the giant freshwater prawn genome. However, only four of these cross-amplified microsatellite primer pairs were reliable and used in this study. Hence, the levels of genetic variability in 12 populations of wild *M. rosenbergii* and one cultured population in Malaysia were evaluated by using 28 microsatellite loci. The number of alleles per locus ranged from 2 to 26 and the total observed heterozygosity ranged from 0.2618 to 0.7265. A high level of polymorphism was also detected in each of the wild *M. rosenbergii* populations by using five RAPD and four LP-RAPD primers which generated 191 bands ranging in molecular weights from 150 bp to 2100 bp in 11 populations. The cross-amplifications of 32 of the 47 newly developed microsatellite primer pairs in nine other prawn species showed the presence of many highly conserved regions among the prawn species tested. However, some of the microsatellite motifs in the nine species tested differed slightly from the originally designed microsatellite loci for *M. rosenbergii*. These newly developed microsatellite loci were used to assess the genetic diversity and relationships of eleven wild *M. rosenbergii* stocks.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai
keperluan ijazah Sarjana Sains

**PEMENCILAN DAN PENCIRIAN LOKUS MICROSATELIT DARIPADA
UDANG GALAH (*MACROBRACHIUM ROSENBERGII*)**

Oleh

SEE LENG MIN

September 2008

Pengerusi: Professor Tan Soon Guan, PhD

Fakulti: Sains

Udang galah, *Macrobrachium rosenbergii* merupakan udang air tawar yang paling popular diternak secara komersial dan menjadi sumber pendapatan penting bagi kebanyakan penternak miskin. Sembilan puluh jujukan berulang mikrosatelit telah berjaya dipencilkan daripada spesies udang galah ini melalui teknik *5' anchored PCR*. Analisis *BLAST* penanda mikrosatelit menunjukkan kesamaan dengan tanda jujukan terungkap (*expressed sequence tags*) dalam spesies akuatik. Enam puluh dua pasang primer mikrosatelit telah direka, di mana 29 adalah mikrosatelit sempurna sementara empat darinya adalah mikrosatelit tidak sempurna atau terganggu dan yang selebihnya adalah mikrosatelit sebatian. Daripada 62 penanda mikrosatelit, 24 pasang primer menunjukkan polimorfik dalam mengamplifikasikan genom udang galah, dengan empat lokus adalah dinukleotid, 16 adalah trinukleotid, tiga adalah tetranukleotid dan satu adalah pentanukleotid. Sembilan pasang primer mikrosatelit daripada kepah (*Perna viridis*) telah berjaya mengamplifikasi-rentas genom udang

galah. Walau bagaimanapun, hanya empat pasang primer mikrosatelit yang digunakan dalam kajian udang galah ini. Oleh itu, variasi genetik dalam 12 populasi spesies liar dan satu populasi ternakan *M. rosenbergii* telah dinilai dengan menggunakan 28 pasang primer mikrosatelit. Bilangan alel per lokus berjulat antara 2 hingga 26 dan jumlah heterozigotiti cerapan berjulat antara 0.2618 hingga 0.7265. Polimorfik yang tinggi telah dikesan dalam setiap populasi liar *M. rosenbergii* dengan menggunakan lima primer RAPD dan empat primer LP-RAPD dan menghasilkan 191 jumlah jalur yang berat molekul berjulat antara 150 bp hingga 2100 bp dalam kesemua 11 populasi. Pengujian amplifikasi-rentas 32 pasang primer daripada 47 pasang primer yang baru direka dalam sembilan jenis spesies udang menunjukkan terdapat kehadiran kawasan terpulihara yang tinggi di kalangan spesies udang galah tersebut. Walau bagaimanapun, sebahagian motif mikrosatelit dalam sembilan spesies udang adalah berbeza daripada lokus mikrosatelit yang direka untuk *M. rosenbergii*. Lokus mikrosatelit yang baru direka telah digunakan untuk mengkaji diversiti genetik dan perhubungan antara 11 populasi liar *M. rosenbergii*.

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I certify that an Examination Committee met on the 8th August 2008 to conduct the final examination of See Leng Min on her Master of Science thesis entitled “Isolation and Characterization of Microsatellite Loci from the Giant Freshwater Prawn (*Macrobrachium rosenbergii*)” in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the student be awarded the Master of Science.

Members of the Examination Committee were as follows:

Nor Aini Mohd. Fadzillah, PhD

Associate Professor
Faculty of Science
Universiti Putra Malaysia
(Chairman)

Siti Khalijah Daud, PhD


Associate Professor
Faculty of Science
Universiti Putra Malaysia
(Internal Examiner)

Jothi Malar Panandam, PhD

Associate Professor
Faculty of Agriculture
Universiti Putra Malaysia
(Internal Examiner)

Siti Azizah Mohd. Nor, PhD

Associate Professor
School of Biological Sciences
Universiti Sains Malaysia
(External Examiner)


HASANA H. GHAZALI, PhD
Professor and Deputy Dean
School of Graduate Studies
Universiti Putra Malaysia

Date: 25 September 2008

This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master of Science.

The members of the Supervisory Committee were as follows:

Tan Soon Guan, PhD

Professor
Faculty of Biotechnology and Biomolecular Sciences
Universiti Putra Malaysia
(Chairman)

Siti Shapor Siraj, PhD

Associate Professor
Faculty of Science
Universiti Putra Malaysia
(Member)

Subha Bhassu, PhD

Lecturer
Institute of Biology Sciences
Universiti Malaya
(Member)



AINI IDERIS, PhD
Professor and Dean
School of Graduate Studies
Universiti Putra Malaysia

Date: 16 OCT 2008

DECLARATION

I hereby declare that the thesis is based on my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UPM or other institutions.



SEE LENG MIN

Date: 5 September 2008

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LIST OF ABBREVIATIONS

AFLP	amplified fragment length polymorphism
AMOVA	analysis of molecular variance
BLAST	basic local alignment search tool
bp	base pair
dATP	2'-deoxyadenosine 5'-triphosphate
DALP	direct amplification of length polymorphism
dCTP	2'-deoxycytidine 5'-triphosphate
dGTP	2'-deoxyguanosine 5'-triphosphate
D _N	genetic distance
dNTP	deoxyribonucleotide
dTTP	2'-deoxythymidine 5'-triphosphate
EST	expressed sequence tag
FAO	Food and Agriculture Organisation
HWE	Hardy-Weinberg equilibrium
<i>lacZ</i>	<i>lac</i> operon that encodes for β-galactosidase
LB	Luria-Bertani
LD	linkage disequilibrium
LP-RAPD	long primer random amplified polymorphic DNA
MgCl ₂	magnesium chloride
NCBI	National Center for Biotechnology Information
PCR	polymerase chain reaction
PIC	polymorphism information content
QTL	quantitative trait loci
RAM	random amplified microsatellite
RAPD	random amplified polymorphic DNA
RFLP	restriction fragment length polymorphism
SNP	Single nucleotide polymorphism
SOC medium	super optimized culture medium
T _A	annealing temperature
TBE	tris borate ethylenediaminetetraacetic acid
T _M	melting temperature
UPGMA	unweighted pair group method with arithmetic averaging
VNTR	variable number of tandem repeats
X-gal	5-bromo-4-chloro-3-indolyl-b-D-galactopyranoside

CHAPTER 1

INTRODUCTION

Freshwater prawn, *Macrobrachium rosenbergii*, or locally known as udang galah is hardy and fast growing, being able to grow in freshwater and low brackish water conditions. This species possesses many biological advantages for commercial culture including attaining maturation in capacity, a relatively large size, and rapid growth rate.

In the 1960's, fishing of giant freshwater prawn was lucrative and there was adequate supply in the country where they exist but at present, increased exploitation and better means of catching has greatly reduced natural stocks and eventually leads to extinction and loss of genetic diversity. Although *M. rosenbergii* has been considered commercially important, biological and genetic information essential to the sustainable management of the resource, such as knowledge of population structure, is lacking. This lack of information has lead to the loss of genetic variability or diversity in the prawn. Comparisons of genetic diversity levels within wild and cultured populations will enable understanding of the effects that intensive culture and small founder populations may have played on levels of genetic diversity in this species.

Assessing genetic variability within and among *M. rosenbergii* is considered important genetic information as it has direct benefit for conserving wild stocks, greater potential for improvement and serves as invaluable resource for different selection criteria, especially when planning breeding or crossbreeding programs. Efficient sampling and utilization of resources would facilitate the determination of genetic variability within and between accessions or varieties. However, the estimation of genetic variation is often limited by the availability of polymorphic genetic markers.

The development of molecular genetic markers has been an important approach toward studying population genetics and dynamics of economically important species. Dominant and codominant markers which have been developed in recent years to characterize population structure and genetic diversity include Random Amplified Polymorphic DNA (RAPD), Random Amplified Microsatellite (RAM) and Microsatellites. In spite of the many types of markers available, the most efficient and effective marker system is microsatellite with its ubiquitous in prokaryotes and eukaryotes genome, and high degree of polymorphism.

1.1 Objectives

The objectives of this study consisted of:

1. Identification and isolation of microsatellite loci in *M. rosenbergii*.
2. Characterization of the microsatellite markers.
3. Assessing the level of genetic variability in *M. rosenbergii* wild stocks.
4. Screening for the cross-amplification of microsatellite primers in nine closely related species.

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